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a nucleic acid containing a mutation indicative of cancer or precancer may be confounded by PCR contaminants in the sample, especially if the detection limits of the assay are near or above the percent contaminants in the sample. The present invention detects PCR contaminants, thus allowing a given PCR reaction to be excluded from analysis on a sample-by-sample basis. Thus, if four separate samples are taken for amplification, each sample is divided into two subsample aliquots, one of which is amplified using chimeric primers, and in the other, the contamination detection sequence primers are used to check for contamination in the aliquot. Therefore, each aliquot of sample for which amplification is sought has its own quality control assay.

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In the claims:

Please cancel claims 1-15 without prejudice, and add new claims 16-37 to read as shown in the attached copy of pending claims.

**REMARKS**

Claims 1-15 were pending in this application and rejected in the Office Action identified above. The specification is amended to correct typographical errors and to amend "Q-PCR" to "quantitative PCR." "Q-PCR" is well known in the art as the shorthand for "quantitative PCR," therefore, Applicant respectfully submits that no new matter is introduced through the amendments in the specification.

Claims 1-15 are canceled without prejudice. New claims 16-37 are added. Upon entry of the present Amendment, claims 16-37 are pending and presented for consideration. Support for new claims 16-37 can be found throughout the specification including claims as originally filed. Applicant respectfully submits that no new matter has been introduced by the present Amendment.

The undersigned wishes to thank the Examiner for her time and courtesy during the telephonic interview that took place on August 23, 2002. The following remarks are

intended to constitute a proper recordation of such interview in accordance with MPEP §713.04, and also to provide a full response to the Office Action mailed on May 9, 2002.

Rejections Under 35 U.S.C. § 112, Second Paragraph

Claims 2 and 8-15 were rejected under 35 U.S.C. § 112, second paragraph, for failing to set forth the subject matter which Applicant regards as his invention. While claims 1-15 are canceled without prejudice, rendering the rejections moot, Applicant hereby addresses Examiner's concerns to the extent they may be applicable in the new claims.

a. The Office Action suggests that it is unclear what is meant by "any contiguous nucleic acid" in the language of claim 2. New claims 17 and 30 now recite that the primer in the control reaction is not complementary to any contiguous nucleic acid sequence in a template or target nucleic acid in the sample. In other words, neither primer recited in claims 17 or 30 is complementary to any continuous stretch of nucleic acid sequence in a target template, although separate parts of the target template, say, two nucleotides here and three nucleotides there, may together be complementary to part or all of the primer sequence.

b. The Office Action rejected claims 8-15 as being vague and indefinite. In particular, the Office Action suggested that the metes and bounds of recited phrase "substantially complementary to said contamination detection sequence" are unclear. Applicant respectfully traverses the rejection and has maintained similar language in new claims 18 and 31. The acceptability of claim language depends on whether one of ordinary skill in the art would understand what is claimed, in light of the specification. MPEP 2173.05(b). The term "substantially" is often used in conjunction with another term to describe a particular characteristic of the claimed invention. MPEP 2173.05(b)(D). The present specification provides that not having substantial sequence specificity means not hybridizing under stringent conditions, which clarifies the metes and bounds of the word "substantially" as recited in the claims. See page 3, lines 26-28. One of ordinary skill in the art, based on the teaching of the present specification, would have been able to appreciate what is meant by the term "substantially" in the new claims

18 and 31. Accordingly, Applicant respectfully submits that the metes and bounds of the claim language at issue are definite under 35 U.S.C. § 112.

c. The Office Action also indicates that claims 8-15 are vague and indefinite because of the claim language “contamination detection sequence” needs better definition. New claim 16 recites a “detection sequence,” and new claim 28 recites a “contamination detection portion.” Applicant respectfully submits that both terms are adequately defined within the respective new claim.

Claims 1-15 were rejected as being incomplete for omitting essential steps regarding how the amplicon is produced and how it relates to the detection of contamination. Applicant believes the Examiner’s concern has been addressed during the interview, and the new claims clarify that the claimed methods relate to detecting contamination by an amplicon from a previous amplification reaction.

In light of the foregoing reasons, Applicant respectfully submits that new claims 16-37 should be free of rejections under 35 U.S.C. § 112, second paragraph.

#### Drawings

The drawings were objected to as not showing the second nucleic acid amplification step. As discussed during the interview, such a step is shown in FIGS. 2B, 2C and 3. Accordingly, Applicant respectfully requests the objection be reconsidered and withdrawn.

#### Rejections Under 35 U.S.C. § 102

Claims 1-4, 7, 8, 10, 11, and 13-15 were rejected under 35 U.S.C. § 102 as being anticipated by WO 91/15601 (hereinafter “Shuldiner”). Again, while claims 1-15 are canceled without prejudice, rendering the rejections moot, Applicant hereby addresses novelty issues as they may be applied to the new claims.

The present invention is different from the method described in Shuldiner in a number of ways. In general, the present invention uses a control amplification reaction to detect false positives resulting from contamination by previous amplicons. In contrast, Shuldiner addresses a different concern, namely, contamination avoidance as opposed to contamination detection. One would not know if there is indeed any contamination following Shuldiner's method.

Shuldiner describes an amplification method that distinguishes cDNA made from endogenous RNA in a sample from contaminating DNA and amplifies only the endogenous RNA sequences (pg. 6, lns. 23-27). Specifically, the method involves using a hybrid primer containing a 5'-unique random sequence in a reverse transcription, resulting in DNA tagged with the unique random sequence (pg. 7, lns. 22-29). Subsequently, a primer specific for the random sequence is used in a PCR reaction conducted in the same sample containing the tagged DNA in order to selectively amplify the tagged DNA (pg. 8, lns. 14-17, and 26-27).

Shuldiner does not teach or suggest a method, as recited in the present claims, for detecting cross-sample contamination in an amplification reaction. As recited by present claim 16, for example, contamination introduced from a previous amplification reaction is detected using at least one primer in a control reaction to amplify a detection sequence incorporated into the amplicon of the previous reaction. Because the primer used in the control reaction does not amplify the target template in the control reaction, a positive result indicates cross-sample contamination. In contrast, the amplification reactions in Shuldiner's method have to be conducted in the same sample, as it avoids DNA contamination by amplifying only sequences derived from endogenous RNA in the same sample (pg. 8, lns. 26-27). Thus, Shuldiner not only fails to teach or suggest detection of contamination, but also fails to teach or suggest detection of cross-sample contamination.

Accordingly, Applicant respectfully submits that Shuldiner does not supply a sufficient basis for a rejection of the pending claims under 35 U.S.C. § 102.

Rejections Under 35 U.S.C. § 103

Claims 5 and 12 were rejected under 35 U.S.C. § 103 (a) as being obvious over Shuldiner in view of U.S. Pat. No. 4,965,188 to Mullis et al. (hereinafter "Mullis").

As described above, Shuldiner does not teach or suggest detection of cross-sample contamination as presently claimed. Mullis describes general methods for amplifying a target nucleic acid sequence in a nucleic acid mixture. *See* Abstract. Its methods use two primers that are complementary to portions of the two strands of a target sequence, but it does not teach or suggest any method for detecting contamination in an amplification reaction. Therefore, the combination of the two references fails to disclose or suggest all the claim elements. Accordingly, Applicant respectfully submits that the 35 U.S.C. § 103 rejections cannot be sustained against the pending claims.


**Summary**

Applicant respectfully requests that the Examiner reconsider the application and claims in light of the foregoing Amendment and Response, and respectfully submits that the pending claims are in condition for allowance. If the Examiner believes that a second telephonic interview would expedite the favorable prosecution of the Application, the undersigned attorney would welcome the opportunity to work with the Examiner toward placing the Application in condition for allowance.

Respectfully submitted,

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## MARKED-UP COPY OF AMENDMENT TO THE SPECIFICATION

The first paragraph on page 2 (lines 1-14) is amended herein as follows:

However, the mere lack of amplification product within the PCR negative control is not determinative of a positive PCR result in a sample in which contamination is rare. This kind of sporadic contamination is especially problematic in an extremely large throughput assay in which 5 to 10 negative controls are run for approximately every 1000 samples. Statistically, the likelihood of sporadic contamination in, for example, 1000 samples will not be detected in only 5 negative controls. Sporadic contamination is also a significant problem when PCR based analyses are performed on heterogeneous (rare event analysis) samples in which a positive result is generated from, for example, 1-5% of the total amplification product present within the sample. Generally, within a PCR based inherited disease diagnostic assay, given the 50% heterogeneity that exists in any genomic DNA sample, a 1-5% increase in signal in a true negative sample would appear as a slight increase in background, but would not indicate a false positive result. However, within an assay involving samples with heterogeneous populations of DNA, a 1-5% positive signal generated by a true negative sample would result in a false positive.

On page 4, please delete the paragraph starting on line 6 and ending on line 23, and substitute the following therefor:

In a preferred embodiment, the amplification reaction is selected from PCR, reverse transcriptase PCR, and ~~Q~~-quantitative PCR. Also in a preferred embodiment, the sample containing nucleic acid to be amplified is a stool sample. A stool sample contains a highly-heterogeneous population of nucleic acids. Human nucleic acids represent a small

portion of the nucleic acid present in stool. More specifically, a stool sample may contain molecular indicia of cancer, specifically colorectal cancer, that occurs as a small subpopulation (typically on the order of about 1% at early stages of cancer or precancer) of the total nucleic acid in the stool. Sensitive assays (which may or may not involve amplification) have been developed to detect such small subpopulations. See, e.g., U.S. Patent No. 5,670,325, incorporated by reference herein. Amplification of a nucleic acid containing a mutation indicative of cancer or precancer may be confounded by PCR contaminants in the sample, especially if the detection limits of the assay are near or above the percent contaminants in the sample. The present invention detects PCR contaminants, thus allowing a given PCR reaction to be excluded from analysis on a sample-by-sample basis. Thus, if four separate samples are taken for amplification, each sample is divided into two subsample aliquots, one of which is amplified using chimeric primers, and in the other, the contamination detection sequence primers are used to check for contamination in the aliquot. Therefore, each aliquot of sample for which amplification is sought has its own quality control assay.

**CLEAN COPY OF ALL PENDING CLAIMS**

1-15. (Canceled)

16. (New) A method for detecting contamination by an amplicon from a previous amplification reaction, said method comprising the steps of:

conducting a control nucleic acid amplification reaction in a sample comprising a nucleic acid template, using at least one primer that is capable of amplifying a detection sequence but not said template, said detection sequence having been incorporated in an amplicon of a previous amplification reaction using at least one chimeric primer comprising said detection sequence at a 5' end of said at least one chimeric primer; and

determining whether said sample has been contaminated by said previous amplification reaction by determining whether said control reaction produces an amplicon.

17. The method of claim 16, wherein said at least one primer in said control reaction is not complementary to any contiguous nucleic acid sequence in said template.

18. The method of claim 16, wherein said at least one primer in said control reaction is substantially complementary to said detection sequence.

19. The method of claim 16, wherein said at least one primer in said control reaction is substantially identical to said detection sequence.

20. The method of claim 16, wherein said at least one primer in said control reaction further comprises an additional sequence 3' to said detection sequence, said additional sequence being specific for a target in said previous amplification reaction.

21. The method of claim 16, wherein said detection sequence is about 20 nucleotides.

22. The method of claim 16, wherein said nucleic acid comprises DNA.

23. The method of claim 16, wherein at least one of said amplification reactions is selected from the group consisting of PCR, quantitative PCR, and reverse-transcriptase PCR.

24. The method of claim 16, wherein said determination step comprises using a sequence-specific nucleic acid probe to capture said amplicon of said control reaction.

25. The method of claim 16, wherein said sample comprises a heterogeneous population of nucleic acids.

26. The method of claim 25, wherein said sample comprises a stool sample.

27. The method of claim 25, wherein said sample comprises a blood sample.

B3  
28. A method for detecting contamination by an amplicon from a previous sample, said method comprising the steps of:

conducting an amplification reaction in a first nucleic acid sample, using at least one chimeric primer comprising a first portion that hybridizes with at least a portion of a target nucleic acid, the amplification of which is desired, and a second, contamination detection portion that does not hybridize with said target nucleic acid;

conducting a control amplification reaction in a second nucleic acid sample, using at least one primer to amplify specifically said contamination detection portion of said chimeric primer; and

determining whether said second sample has been contaminated by an amplicon from said first sample by determining whether said control reaction produces an amplicon.

29. The method of claim 28, wherein said second portion is 5' to said first portion in each of said at least one chimeric primers.

30. The method of claim 28, wherein said at least one primer in said control reaction is not complementary to any contiguous nucleic acid sequence in any target nucleic acid in said second sample.

31. The method of claim 28, wherein said at least one primer used in said control reaction is substantially complementary to said contamination detection portion.

32. The method of claim 28, wherein said at least one primer used in said control reaction is substantially identical to said contamination detection portion.
33. The method of claim 28, wherein at least one of said amplification reactions is selected from the group consisting of PCR, quantitative PCR, and reverse-transcriptase PCR.
34. The method of claim 28, wherein said samples comprise a heterogeneous population of nucleic acids.
35. The method of claim 34, wherein said samples comprise a stool sample.
36. The method of claim 34, wherein said samples comprise a blood sample.
37. The method of claim 28, wherein said contamination detection portion is about 20 nucleotides.